# *Mycobacterium chimaera* in Heater–Cooler Units in Denmark Related to Isolates from the United States and United Kingdom

Erik Svensson, Elsebeth Tvenstrup Jensen, Erik Michael Rasmussen, Dorte Bek Folkvardsen, Anders Norman, Troels Lillebaek

*Mycobacterium chimaera* was present at high rates (≥80%) in heater–cooler units (HCUs) from all 5 thoracic surgery departments in Denmark. Isolates were clonal to HCU-associated isolates from the United States (including some from patients) and United Kingdom. However, *M. chimaera* from 2 brands of HCU were genetically distinct.

Based on reports from 2015 (*1*,*2*), the European Centre for Disease Prevention and Control issued a Rapid Risk Assessment alert on April 30, 2015, associating invasive cardiovascular infections with *Mycobacterium chimaera* in water tanks of heater–cooler units (HCUs) used during open-chest heart and vascular surgery (*3*). Subsequently, additional cases from Europe (*4*) and the United States potentially associated with HCUs have been described (*5*–*7*). Preliminary data indicate that the isolates from the patients, the HCUs in hospitals, and the HCUs at the manufacturer are similar (*8*). The aim of this study was to determine *M. chimaera* prevalence in Denmark HCUs and, if present, phylogenetically characterize and quantify the strains.

## The Study

Statens Serum Institut, the Danish Patient Safety Authority, and the Danish Medicines Agency decided to investigate all the HCUs in Denmark. Approval from a human or animal research ethics board was not required to conduct this study. The infection prevention control units and thoracic surgery department staff from 5 local hospitals were instructed to collect water and biofilm samples from the HCUs and send them to Statens Serum Institut for testing. In brief, a water culture method adapted for low concentrations of mycobacteria with high concentrations of contaminants was used, and mycobacterial isolates were identified by internal transcribed spacer sequencing (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/3/16-1941-Techapp1.pdf). One *M. chimaera* isolate from each thoracic surgery department and one unrelated patient isolate were subjected to whole-genome sequencing (WGS). A total of 10 million paired-end Illumina sequencing reads (Illumina Denmark ApS, Copenhagen, Denmark) were deposited in the European Nucleotide Archive under study number PRJEB18427.

*M. chimaera* was found in 18/21 (86%) HCUs, representing all 5 thoracic surgery departments in Denmark (Table). Four sites used the Sorin 3T HCU (Sorin Group, Arvada, CO, USA); 14/16 (88%) units contained *M. chimaera*. One site used Maquet brand HCUs (Maquet, Wayne, NJ, USA), 4/5 (80%) units contained *M. chimaera*. The strain *M. gordonae* was found irregularly throughout the HCUs (Table). Both water and biofilm samples could be cultured and were equally effective for the detection of mycobacteria. We used the filter culture method for quantitative analysis purposes and for a simpler workflow. However, the quantitative culture results were poor quality because the analytic sensitivity was low and many samples were heavily contaminated (Table; online Technical Appendix Figure).

WGS analysis (Figure) showed that the 4 isolates from the Denmark Sorin 3T HCUs were nearly identical (<3 single nucleotide polymorphisms [SNPs]). Conversely, the isolate collected from the Maquet HCU was genetically distinct, showing 47–49 SNP differences compared with the isolates from the Sorin 3T HCUs. The unrelated patient isolate was not closely related to the HCU isolates (30–37 SNP differences).

We compared the sequences we collected with 31 other sequenced *M. chimeaera* isolates previously collected (2009–2016) and available in the European Nucleotide Archive (http://www.ebi.ac.uk/ena). This dataset comprised 3 non–HCU-associated patient isolates from Ireland; 9 Sorin 3T HCU isolates and 11 HCU-associated patient isolates from Pennsylvania and Iowa, USA (*6*); and 8 HCU water sample isolates from the United Kingdom (accession nos. PRJNA294775, PRJNA344472, PRJNA345021, and PRJNA324238 for the 4 groups, respectively). Unexpectedly, the *M. chimaera* sequences from the Denmark Sorin 3T HCUs were nearly identical to the isolates from the United States and United Kingdom (median difference 3 SNPs; interquartile range [IQR] 1–5 SNPs) and were similar to all Sorin 3T-associated patient isolates (median difference 6 SNPs; IQR 3–9 SNPs). We saw a distinctly closer relationship between the isolates from Denmark Sorin 3T HCUs and the isolates from UK and US HCUs than between Denmark Sorin 3T

Author affiliation: Statens Serum Institut, Copenhagen, Denmark

DOI: http://dx.doi.org/10.3201/eid2303.161941

**Table.** Identification of *Mycobacterium* spp. from water and biofilm samples taken from heater–cooler units from 5 heart surgery centers, Denmark, July–October 2015*

| Heater–cooler unit | Water sample results | Biofilm sample results | Quantitative culture, CFU/L† |
|---|---|---|---|
| A1 | *M. chimaera* | NA | 100 |
| A2 | *M. chimaera* | NA | Mold |
| A3 | *M. chimaera* | NA | 60 |
| B1 | *M. chimaera* | NA | Mold |
| C1 | *M. chimaera* | *M. chimaera* | 0 |
| C2 | *M. gordonae* | *M. gordonae* | 9 |
| C3 | *M. chimaera* | *M. chimaera* | Mold |
| C4 | *M. gordonae* | *M. gordonae* | 57 |
| D1 | *M. chimaera* | *M. chimaera* | >1,000 |
| D2 | *M. chimaera* | NA | Mold |
| D3 | *M. chimaera* | *M. chimaera* | Mold |
| D4 | *M. chimaera* | *M. chimaera* | >1,000 |
| D5 | *M. chimaera* | *M. chimaera* | Mold |
| D6 | *M. chimaera* | NA | Mold |
| D7 | *M. chimaera* | *M. chimaera* | Mold |
| D8 | *M. chimaera* | *M. chimaera* | Mold |
| E1 | *M. gordonae* | *M. chimaera* | 0 |
| E2 | *M. chimaera* | *M. chimaera*, *M. gordonae* | 0 |
| E3 | *M. chimaera* | *M. chimaera* | 0 |
| E4 | *M. chimaera* | *M. chimaera*, *M. gordonae* | 300 |
| E5 | No growth | NA | 0 |

*NA, not available.
†Bacterial concentration of the original sample.

HCUs and the unrelated Denmark or Ireland patient isolates or the Denmark Maquet HCU isolate (Figure).

Overall, the 32 isolates associated with the Sorin 3T HCUs (online Technical Appendix Table) were found to have 15 common SNPs and 0–18 SNP differences between any 2 isolates (median difference 5 SNPs; IQR 3–8 SNPs). These findings support the conclusion by Haller et al. that *M. chimaera* from the Sorin 3T HCUs have a common source (*8*). The *M. chimaera* sequences from the UK HCU water samples were genetically nearly identical to the US and Denmark isolates; we therefore conclude that the UK isolates also originated from Sorin 3T HCUs.

No patients with *M. chimaera* infections associated with open-chest surgery have been suspected or detected

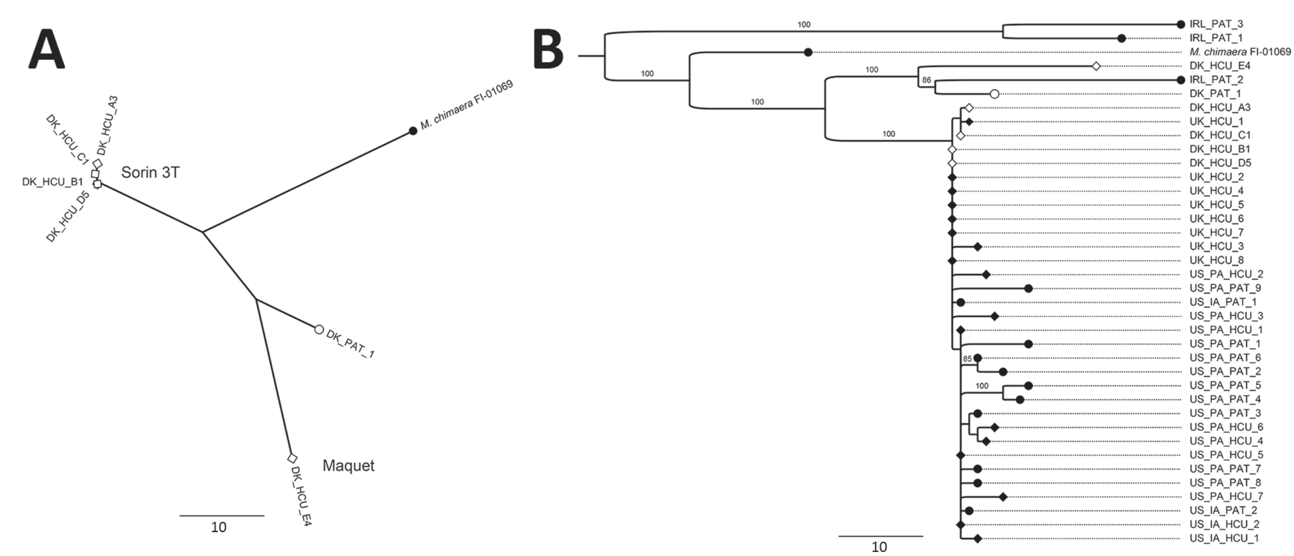



**Figure.** Maximum parsimony phylogenies showing the relationship between *Mycobacterium chimaera* isolates found in patients (circles) and heater–cooler units (HCUs; diamonds) in Denmark. *M. chimaera* strain FI-0169 (accession no. PRJNA356276) was included for reference. A) Tree showing isolates from Sorin 3T (Sorin Group, Arvada, CO, USA) and Maquet (Maquet, Wayne, NJ, USA) HCUs and a non–HCU-associated isolate from a patient (PAT) in Denmark (DK). B) Phylogenetic tree comparing isolates from Denmark to 31 isolates collected in 3 other countries (Ireland [IRL], United Kingdom, and United States) and retrieved from the European Nucleotide Archive (http://www.ebi.ac.uk/ena). Isolates from Denmark are indicated by open symbols and isolates from other countries by solid symbols. Branch values indicate percentwise bootstrap support (only >70% support is shown), based on 100 replicates. IA, Iowa; PA, Pennsylvania. Scale bars represent a difference of 10 single-nucleotide polymorphisms.

in Denmark. Searching the International Reference Laboratory of Mycobacteriology database, which includes all mycobacteria cultures in Denmark, from 1991 to 2016, we found no records of *M. avium* complex isolates from patients that had an open-chest operation.

Following our findings, 1 thoracic surgery department decided to keep the HCUs in the operating theater but encased them in housings with separate ventilation. Two departments were unable to take the HCUs out of the theaters but decided to move the HCUs as distant as possible from the patients and decontaminate more frequently. Two of the departments had their HCUs outside the operating room already and therefore kept their policies regarding HCUs.

## Conclusions

We found that *M. chimaera* was present in most HCUs in Denmark. Isolates from Sorin 3T brand HCUs were identical to the HCU isolates from the United States and the United Kingdom, and thus they appear to have the same origin. Because all 5 of the thoracic surgery departments in Denmark had contaminated HCUs and because mycobacterial contamination has been reported in multiple published studies during 2015–2016 (*4*–*6*), we find it likely that most Sorin 3T HCUs made in the past 8–10 years potentially are contaminated by the same *M. chimaera* strain. In addition, because 80% of the Maquet HCUs also contained *M. chimaera*, although phylogenetically different from the Sorin 3T strains, we suggest mycobacterial contamination might be a general problem for HCUs.

Dr. Svensson specializes in clinical microbiology and works at Statens Serum Institut in Denmark as Technical Director for Diagnostics at the International Reference Laboratory of Mycobacteriology. His research interests are molecular epidemiology, mycobacteria, and pharmacodynamics of antibiotics.

## References

1. Sax H, Bloemberg G, Hasse B, Sommerstein R, Kohler P, Achermann Y, et al. Prolonged outbreak of *Mycobacterium chimaera* infection after open-chest heart surgery. Clin Infect Dis. 2015;61:67–75. http://dx.doi.org/10.1093/cid/civ198
2. Kohler P, Kuster SP, Bloemberg G, Schulthess B, Frank M, Tanner FC, et al. Healthcare-associated prosthetic heart valve, aortic vascular graft, and disseminated *Mycobacterium chimaera* infections subsequent to open heart surgery. Eur Heart J. 2015;36:2745–53. http://dx.doi.org/10.1093/eurheartj/ehv342
3. European Centre for Disease Prevention and Control. Invasive cardiovascular infection by *Mycobacterium chimaera*. 2015 Apr 30 [cited 2016 Jul 15]. http://ecdc.europa.eu/en/publications/Publications/mycobacterium-chimaera-infection-associated-with-heater-cooler-units-rapid-risk-assessment-30-April-2015.pdf
4. European Centre for Disease Prevention and Control. Invasive cardiovascular infection by *Mycobacterium chimaera* associated with the 3T heater-cooler system used during open-heart surgery. 2016 Nov 18 [cited 2016 Nov 30]. http://ecdc.europa.eu/en/publications/Publications/RRA-mycobacterium-chimaera-November-2016.pdf
5. Public Health England. Infections associated with heater cooler units used in cardiopulmonary bypass and ECMO: information for healthcare providers in England. 2015 Nov 5 [cited 2016 Oct 17]. https://www.gov.uk/government/publications/infections-associated-with-heater-cooler-units-used-in-cardiopulmonary-bypass-and-ecmo
6. Perkins KM, Lawsin A, Hasan NA, Strong M, Halpin AL, Rodger RR, et al. Notes from the field: *Mycobacterium chimaera* contamination of heater-cooler devices used in cardiac surgery–United States. MMWR Morb Mortal Wkly Rep. 2016;65:1117–8. http://dx.doi.org/10.15585/mmwr.mm6540a6
7. Tan N, Sampath R, Abu Saleh OM, Tweet MS, Jevremovic D, Alniemi S, et al. Disseminated *Mycobacterium chimaera* infection after cardiothoracic surgery. Open Forum Infect Dis. 2016;3:ofw131. http://dx.doi.org/10.1093/ofid/ofw131
8. Haller S, Höller C, Jacobshagen A, Hamouda O, Abu Sin M, Monnet DL, et al. Contamination during production of heater-cooler units by *Mycobacterium chimaera* potential cause for invasive cardiovascular infections: results of an outbreak investigation in Germany, April 2015 to February 2016. Euro Surveill. 2016;21:30215. http://dx.doi.org/10.2807/1560-7917.ES.2016.21.17.30215

Address for correspondence: Erik Svensson, International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark; email: esn@ssi.dk

Article DOI: http://dx.doi.org/10.3201/eid2303.161941

# *Mycobacterium chimaera* in Heater–Cooler Units in Denmark Related to Isolates from the United States and United Kingdom

## Technical Appendix

### Sampling

Statens Serum Institut, the Danish Patient Safety Authority, and the Danish Medicines Agency decided to investigate all Denmark heater–cooler units (HCUs) and to increase the awareness of symptoms of *M. chimaera* infections in patients who had undergone open chest heart surgery previously. The 5 local hospital's infection prevention and control units, surgeons, perfusionists, and other relevant staff were specifically recruited, participated in network meetings, and were provided with instructions on how to sample water and biofilm from the HCUs. In return, Statens Serum Institut and the Danish Medicines Agency gained helpful information on the number and models of HCUs used throughout the country.

Samples were collected from all the HCUs located in thoracic surgery departments (n = 5) throughout the country. The departments were instructed to submit 1 L of water from each tank for the detection of planktonic mycobacteria, as well as provide swabs from the inner surfaces of tube mouths from each HCU for the detection of mycobacteria in the biofilm.

### Culture

Each 1-L water sample was divided into 3 aliquots. All 3 were concentrated by centrifugation at 3,000 × *g* in a Sorvall RC 6 Plus Centrifuge (Thermo Fisher Scientific, Waltham, MA, USA), followed by removal of the supernatant. The sediments were decontaminated separately using N-acetyl-cysteine and 2% NaOH (Mycoprep, Becton Dickinson, Sparks, MD, USA) for 10 min, neutralized with phosphate buffer (pH 6.8), and centrifuged for 15 min at 3,700 rpm in a Heraeus Multifuge 3SR+ (Thermo Fisher Scientific Waltham, MA, USA). Then supernatants were removed, leaving ≈1.5 mL of sediment. Two of the 3 sediment aliquots were used for qualitative culture and the remaining aliquot for quantitative culture on a filter. A portion from each of the 2 aliquots was inoculated separately into a mycobacterium growth indicator tube (Becton Dickinson, Sparks, MD, USA) and a Löwenstein-Jensen tube (SSI Diagnostica, Copenhagen, Denmark). The remaining portion of the 2 aliquots were kept in the refrigerator as a backup if the culture was contaminated. The third aliquot was frozen for later quantitative culture. To quantitate, the frozen aliquots were thawed and diluted with 100 mL of phosphate buffer and passed through a MicroFunnel 300 black membrane filter with a 0.45-µm pore size (Pall Corporation, Westborough, MA, USA). The filter was put on Middlebrook 7H11 agar and incubated at 30°C for 8 weeks.

## Identification

The growing mycobacteria were identified using GenoType CM line probe assay (Hain Lifescience, Nehren, Germany) as a screening test. If the test result was positive for *M. intracellulare*, we then performed internal transcribed spacer (ITS) sequencing (*1*,*2*).

Five microliters of DNA (extracted from cells with heat inactivation and sonication) was amplified and hybridized with GenoType CM kit (Hain Lifescience) according to the instructions from the manufacturer. Initial PCR for ITS sequencing was done with a 5 µL sample of DNA in a 50 µL reaction containing 5 µL 10× PCR buffer, 10 µL 5× Q-solution, 2 µL 25 mM $MgCl_2$, 1 µL 10 µM primer ITS_F, 1 µL 10 µM primer ITS_R (*1*,*2*), 0.25 µL HotStarTaq (HotStarTaq Master Mix Kit; QIAGEN, Hilden, Germany), and water (DNase, RNase, and protease free). The thermal profile involved a denaturation step of 95°C for 15 min; 40 cycles of 95°C for 60 s, 59°C for 60 s, and 72°C for 60 s; a final extension at 72°C for 10 min; and a cool down at 4°C. The sequencing was performed using the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. Sequence analysis was performed using Sequencing Analysis Software v.5.3.1 (Applied Biosystems).

## Whole-Genome Sequencing

In brief, 1 mL of a culture enriched in Dubos broth (SSI Diagnostika, Hilleroed, Denmark) was centrifuged at 13,000 rpm for 10 min, the supernatant removed, and the pellet resuspended in 300 µL TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 8.0). The resuspended pellets were heated at 80°C for 20 min to kill the cells and incubated with 50 µL of 10 mg/mL lysozyme for 24 h at 37°C on a Thermomixer C (Eppendorf, Hamburg, Germany). Then 70 µL of 10% sodium dodecyl sulfate and 5 µL of 10 mg/mL proteinase K were added, and the mixture was incubated for 10 min at 65°C. Next, 100 µL of 5 M NaCl and 100 µL of N-cetyl-N,N,N-trimethyl ammonium bromide/NaCl was added. The tubes were vortexed briefly and incubated for 10 min at 65°C. An equal volume of chloroform/isoamyl alcohol (24:1, vol/vol) was added, the mixture was vortexed for 10 s, and centrifuged at 13,000 rpm for 10 min. Afterwards, 0.6 volume of isopropanol was added to the supernatant to precipitate the DNA. The tubes were left overnight at –20°C and centrifuged for 15 min; the pellet was washed once with 70% ethanol, air-dried, and redissolved in 20 µL of TE buffer.

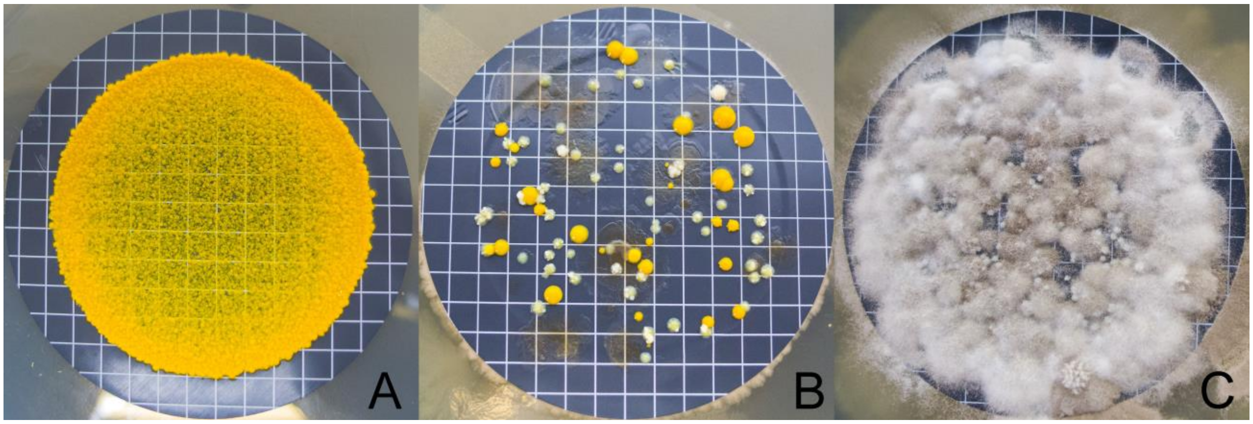
DNA was normalized to a concentration of 0.5 ng/µL using a Biomek NX Lab Automation Workstation (Beckman Coulter, Indianapolis, IN, USA). DNA libraries were generated using a Nextera XT DNA preparation kit (Illumina, Inc., San Diego, CA, USA) according to the manufacturer's instructions but diluted an extra 4-fold before being loaded on to the NextSeq 500 platform (Illumina Inc.) with 2×150 bp paired-end chemistry.

Paired-end Illumina reads were end-trimmed (base-quality >Q3; minimum end-trimmed length >35 bp) and filtered using Trimmomatic (http://www.usadellab.org/cms/?page=trimmomatic), and mapped to the 5.8 Mbp *M. chimera* FI-01069 chromosome sequence (accession no. PRJNA324238) using the Burrows-Wheeler Alignment tool bwa (http://bio-bwa.sourceforge.net/) (see Technical Appendix Table for an overview of mapped samples used in this study). Variant calling was performed with SAMtools and BCFtools (http://www.htslib.org/) (*3*). Single-nucleotide polymorphisms (SNPs) were filtered according to the following criteria: having a phred-scaled variant calling quality exceeding 20 (http://gatkforums.broadinstitute.org/gatk/discussion/4260/phred-scaled-quality-scores) and being supported by ≥5 reads with a frequency of 85% in ≥1 sample. However, to minimize the influence of horizontal gene transfer, regions in which ≥3 SNPs occurred together within 100 bp were excluded from the analysis. For individual samples, SNPs were considered as fixated if they were supported by ≥3 reads with a SNP-frequency above 70%, or otherwise called the reference, unless present in ≥5 other samples. Phylogenetic comparison of concatenated SNPs was performed with the Seaview program (*4*) by using the built-in maximum parsimony function (Dnapars, http://evolution.genetics.washington.edu/phylip/doc/dnapars.html). Branch support values were calculated using 100 bootstrap replicates. A distance matrix was created by importing the generated SNP alignment in FASTA format (http://zhanglab.ccmb.med.umich.edu/FASTA/) into the statistics software RStudio (v1.0.44) (https://www.rstudio.com/) using the Analyses of Phylogenetics and Evolution package (http://ape-package.ird.fr/) to calculate pairwise sample distances.

**Technical Appendix Table.** Overview of whole-genome sequencing of *Mycobacterium chimaera* isolated from heater–cooler units (HCUs) and patients, Denmark, July–October 2015*

| Sample name | Study accession no. | Run accession no. | Country | Collection date | Isolation source | No. trimmed reads | Reads mapped, % | FI-01069 coverage, X |
|---|---|---|---|---|---|---|---|---|
| DK_HCU_A3 | PRJEB18427 | ERR1744899 | DK | 2015 Jul 15 | HCU (3T) | 422,370 | 88.3 | 9 |
| DK_HCU_B1 | PRJEB18427 | ERR1744900 | DK | 2015 Aug 8 | HCU (3T) | 2,916,088 | 88.7 | 61 |
| DK_HCU_C1 | PRJEB18427 | ERR1744901; ERR1744902 | DK | 2015 Jul 28 | HCU (3T) | 2,011,606 | 87.7 | 41 |
| DK_HCU_D5 | PRJEB18427 | ERR1744903 | DK | 2015 Oct 1 | HCU (3T) | 1,552,694 | 85.9 | 32 |
| DK_HCU_E4 | PRJEB18427 | ERR1744904 | DK | 2015 Oct 1 | HCU (Maquet) | 1,850,980 | 87.4 | 39 |
| DK_PAT_1 | PRJEB18427 | ERR1744905 | DK | 2015 Jul 13 | BAL | 1,034,652 | 90.0 | 23 |
| FI-01069 | PRJEB18427 | ERR1744906 | IT | 2001 | BAL | 1,636,840 | 96.1 | 37 |
| IRL_PAT_1 | PRJNA294775 | SRR2338871 | IRL | 2009 | Sputum | 3,466,168 | 92.5 | 52 |
| IRL_PAT_2 | PRJNA294775 | SRR2338873 | IRL | 2013 | Sputum | 4,040,276 | 96.4 | 81 |
| IRL_PAT_3 | PRJNA294775 | SRR2338874 | IRL | 2014 | BAL | 3,334,536 | 90.3 | 70 |
| UK_HCU_WAT_1 | PRJNA324238 | SRR4068047 | UK | 2015 | HCU (Unknown) | 2,122,042 | 90.3 | 46 |
| UK_HCU_WAT_2 | PRJNA324238 | SRR4119603 | UK | 2015 | HCU (Unknown) | 2,642,246 | 92.5 | 39 |
| UK_HCU_WAT_3 | PRJNA324238 | SRR4119606 | UK | 2015 | HCU (Unknown) | 3,459,964 | 87.2 | 49 |
| UK_HCU_WAT_4 | PRJNA324238 | SRR4119612 | UK | 2015 | HCU (Unknown) | 2,753,690 | 92.6 | 41 |
| UK_HCU_WAT_5 | PRJNA324238 | SRR4119617 | UK | 2015 | HCU (Unknown) | 2,762,350 | 92.3 | 41 |
| UK_HCU_WAT_6 | PRJNA324238 | SRR4119619 | UK | 2015 | HCU (Unknown) | 2,604,538 | 93.0 | 39 |
| UK_HCU_WAT_7 | PRJNA324238 | SRR4119623 | UK | 2015 | HCU (Unknown) | 7,623,682 | 88.2 | 104 |
| UK_HCU_WAT_8 | PRJNA324238 | SRR4119656 | UK | 2015 | HCU (Unknown) | 5,384,290 | 91.2 | 79 |
| US_PA_HCU_1 | PRJNA344472 | SRR4295156 | USA (PA) | 2015 Jul 25 | HCU (3T) | 2,514,644 | 94.5 | 90 |
| US_PA_HCU_2 | PRJNA344472 | SRR4295157 | USA (PA) | 2015 | HCU (3T) | 1,996,066 | 88.0 | 65 |
| US_PA_PAT_1 | PRJNA344472 | SRR4295158 | USA (PA) | 2015 May 1 | Human | 2,472,668 | 94.5 | 90 |
| US_PA_HCU_3 | PRJNA344472 | SRR4295159 | USA (PA) | 2015 Oct 30 | HCU (3T) | 2,861,298 | 90.3 | 97 |
| US_PA_PAT_2 | PRJNA344472 | SRR4295160 | USA (PA) | 2015 Sep 11 | Human | 2,934,922 | 94.7 | 106 |
| US_PA_PAT_3 | PRJNA344472 | SRR4295161 | USA (PA) | 2015 Nov 6 | Human | 2,184,438 | 91.1 | 77 |
| US_PA_PAT_4 | PRJNA344472 | SRR4295162 | USA (PA) | 2016 Feb 3 | Human | 2,636,322 | 91.4 | 94 |
| US_PA_PAT_5 | PRJNA344472 | SRR4295163 | USA (PA) | 2016 Mar 21 | Human | 2,112,644 | 90.9 | 76 |
| US_PA_HCU_4 | PRJNA344472 | SRR4295164 | USA (PA) | 2015 | HCU (3T) | 3,176,156 | 90.0 | 108 |
| US_PA_HCU_5 | PRJNA344472 | SRR4295165 | USA (PA) | 2015 | HCU (3T) | 3,143,036 | 93.6 | 110 |
| US_PA_HCU_6 | PRJNA344472 | SRR4295166 | USA (PA) | 2015 | HCU (3T) | 2,731,838 | 94.6 | 99 |
| US_PA_PAT_6 | PRJNA344472 | SRR4295167 | USA (PA) | 2015 Jun 15 | Human | 3,030,308 | 93.9 | 109 |
| US_PA_PAT_7 | PRJNA344472 | SRR4295168 | USA (PA) | 2014 Jul 24 | Human | 2,595,368 | 94.7 | 94 |
| US_PA_PAT_8 | PRJNA344472 | SRR4295169 | USA (PA) | 2014 May 20 | Human | 2,309,386 | 94.4 | 84 |
| US_PA_HCU_7 | PRJNA344472 | SRR4295170 | USA (PA) | 2015 Aug 19 | HCU (3T) | 2,139,370 | 86.8 | 72 |
| US_PA_PAT_9 | PRJNA344472 | SRR4295171 | USA (PA) | 2015 Jan 28 | Human | 2,115,298 | 87.9 | 72 |
| US_IA_PAT_1 | PRJNA345021 | SRR4324922 | USA (IA) | 2015 Nov 11 | Blood | 1,230,194 | 95.4 | 44 |
| US_UA_PAT_2 | PRJNA345021 | SRR4324923 | USA (IA) | 2016 Feb 8 | Blood | 2,087,698 | 92.7 | 89 |
| US_IA_HCU_1 | PRJNA345021 | SRR4324924 | USA (IA) | 2016 Jan 28 | HCU (3T) | 1,173,912 | 87.0 | 43 |
| US_IA_HCU_2 | PRJNA345021 | SRR4324925 | USA (IA) | 2016 Apr 13 | HCU (3T) | 1,524,522 | 93.5 | 72 |

*DK, Denmark; HCU, heater–cooler unit; IA, Iowa; IRL, Ireland; IT, Italy; PA, Pennsylvania; PAT, patient; WAT, water.




**Technical Appendix Figure.** Quantitative culture of filtered water from heater–cooler units. The images show filters with (A) >1,000 CFU/L, (B) 300 CFU/L with some contamination from other bacteria, and (C) heavy overgrowth of mold.

**References**

1. De Smet KA, Brown IN, Yates M, Ivanyi J. Ribosomal internal transcribed spacer sequences are identical among *Mycobacterium avium*-intracellulare complex isolates from AIDS patients, but vary among isolates from elderly pulmonary disease patients. Microbiology. 1995;141:2739–47. PubMed http://dx.doi.org/10.1099/13500872-141-10-2739

2. Frothingham R, Wilson KH. Sequence-based differentiation of strains in the *Mycobacterium avium* complex. J Bacteriol. 1993;175:2818–25. PubMed http://dx.doi.org/10.1128/jb.175.10.2818-2825.1993

3. Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics. 2011;27:2987–93. PubMed http://dx.doi.org/10.1093/bioinformatics/btr509

4. Gouy M, Guindon S, Gascuel O. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol. 2010;27:221–4. PubMed http://dx.doi.org/10.1093/molbev/msp259